CYTOTOXIC POTENTIALS AND PHYTOCONSTITUENTS PROFILING OF *BLEPHARIS EDULIS* (FORSSK.) PERS. USING UHPLC/Q-TOF-MS-MS

Taha Abdel Maboud[*1], Mostafa M. Hegazy[1], Abdel Salam Ebrahim[1] and Lotfy D. Ismail[1].

[1] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

***Corresponding author:** TahaKedra.2@azhar.edu.eg

ABSTRACT

Recently, the demand for discovery of safe anticancer drugs from nature has been extensively increased to avoid cancer chemotherapy side effects. *Blepharis edulis* (Forssk.) pers (Acanthaceae) is a perennial herb growing in Egypt with many traditional and pharmacological activities. The in *vitro* assay as well as identification of phytoconstituents in plant samples by LC/MS/MS spectrometry has reserve time and efforts for rapid screening and identification of biologically active compounds in plants. Herein, we investigated the cytotoxic activity of different fractions of *Blepharis edulis* methanolic extract using Sulforhodamine B stain (SRB) against three cancer cell lines; Hepatocellular carcinoma (HepG-2), human colon carcinoma (HCT-116), and human breast adenocarcinoma (MCF-7) cell lines. The results revealed that the *n*-butanol fraction showed the most potent cytotoxic activity against the three tested cancer cell lines with CC_{50} values 9.12 ± 0.92, 6.79 ± 0.65 and 4.19 ± 0.51 against MCF-7, HCT-116 and HepG2 cell lines, respectively. Moreover, the high resolution the ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF-MS-MS) metabolomic analysis for the *n*-butanol fraction as the most active sample has been achieved for the first time. A total of 3، compound of different classes including flavonoids, aromatic aldehydes, aromatic acids and alkaloids, have been identified from *Blepharis edulis*.

Keywords: *Blepharis edulis*, Acanthaceae, cytotoxic activity, UHPLC/Q-TOF-MS-MS.

Introduction

Cancer is a major public health problem all over the world and one of the major causes of death in developing countries, together with cardiac and cerebrovascular diseases (Ueda, Tezuka et al. 2002). Despite the great progress of cancer chemotherapy, the resulted side effects are still provoked and often severe causing patient incompliance (Schirrmacher 2019).Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanisms of action (George, Bhalerao et al. 2010). Over 50% of drugs used in clinical trials for anticancer activity have been isolated from natural sources or are related to them (George, Bhalerao et al. 2010). *Blepharis* is the largest genus of Acanthaceae comprises about 129 perennial herbs plants species widely distributed in India, Pakistan, Iran as well as Egypt (Vijayalakshmi and Kripa 2016). The plants are found in seasonally dry to arid habitats. Many species of *Blepharis* have been used in folk medicine as analgesic, in treatment of inflammation, and microbial infection and male impotence. There are many traditional uses for *B. edulis* as antiarthritic, antibacterial, antifungal, antioxidant, potent aphrodisiac and antispasmodic activities (Mundla and Sitaram 2013). Moreover, *B. edulis* is among the ingredients used in a herbal composition for vigor, vitality and general health tonic (Pande and Pathak 2009). Further, *B. edulis* revealed many biological activities as antioxidant (Ashour 2012, Mahboubi, Haghi et al. 2013), anti-inflammatory (kumar Duvey and Chowdhary 2016), aphrodisiac (Mathur and Sundaramoorthy 2009, Singh, Ali et al. 2013), antispasmodic, bronchodilator, antiplatelet aggregation (Saqib, Janbaz et al. 2012), antidiabetic, antihyperlipidemic (Kant, Dua et al. 2018), anti-leishmanial (de Sousa, Lima et al.), antibacterial and anti-fungal activities (Keymanesh, Hamedi et al. 2009). Recent investigations of genus *Blepharis edulis* revealed the presence of many phytochemical classes as, phenols, terpenoids, saponins, tannins, xanthoproteins. Alkaloids and flavonoid (Chatterjee, Sharma et al. 1990, HS and Leelavathi 2015). Currently, ultra-high performance liquid chromatography (UHPLC) combined with High-resolution mass techniques allows fast fingerprinting and identification of plant metabolomics including several flavonoids, coumarins, phenolic acids and terpenes in biological samples, medicinal plants and food (Echiburu-Chau, Pastén et al. 2017, Simirgiotis, Quispe et al. 2017). In the present work we aimed to profile the phytoconstituents of *B. edulis* most active cytotoxic fraction by the high resolution UHPLC/Q-TOF-MS-MS.

Material and methods

Experimental section

The analysis was carried out using an ExionLC Triple TOF 5600+ system (Sciex) operated at 40 °C and equipped with a Xbridge C-18 column (Waters, 3.5 µm, 2.1 x 50 mm), and precolumn (Phenomenex In-Line filter disks, 0.5 µm x 3.0 mm).

Plant material

Blepharis edulis aerial parts were collected at March 2017 from Al-Nakhl area in North Sinai, and kindly identified by Prof. Mohamed S. Tantawy, Professor of Plant Taxonomy, Faculty of Science, Ain-shams University. A voucher herbarium specimen had been deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azher University, Nasr City, Cairo, Egypt.

Extraction and fractionation

Air-dried powdered aerial parts of *B. edulis* (980 g) were subjected to exhaustive extraction by percolation with aqueous 70 % MeOH (3 x 4 L). The combined methanolic extracts were concentrated under vacuum at 40 °C until dryness to obtain 130 g viscous residue. The dried extract was suspended in distilled water (800 ml) and fractionated with different solvents to obtain *n*-hexane (10 g), dichloromethane (4 g), ethyl acetate (2 g), *n*-butanol (20 g), and (80 g) aqueous fractions.

Assay of cytotoxic activity

Cell viability screening was carried out against three cancer cell lines; hepatocellular carcinoma (HepG-2), human colon carcinoma (HCT-116), and human breast adenocarcinoma (MCF-7) cell lines to determine 50% Cytotoxic Concentration (CC_{50}). The cytotoxic activity was measured quantitatively using Sulforhodamine B stain (SRB) assay protocol (Skehan, Storeng et al. 1990, Vajrabhaya and Korsuwannawong 2018). Briefly, the cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) media (Lonza group) supplemented with 200 *m*M of L-glutamine and 10% of fetal bovine serum (FBS). The test fractions as well as astandard drug were dissolved in a mixture of Dimethyl Sulfoxide and Dulbecco′s Modified Eagle′s Medium (DMEM) with ratio 4:100 (v/v), respectively. An initial dose of (1 mg/ml) was tested on different cell lines and sub sequenced by five more dilutions using two-fold dilution factor to obtain (0, 6.25, 12.5, 25, 50 and 100 µg/mL). Cells were seeded with a concentration of (6×10^4 cell/ml) for 24 hours in the flat bottom 96 well plates and incubated at 37 °C with 5% CO_2 until semi confluent cell layer was obtained then, treated with 100 µl of each of serially diluted compounds. After 48 hours, the anticancer activity of the tested samples was measured quantitatively by ELISA microplate reader at wave length 520 nm according to SRB assay protocol. All experiments were carried out in triplicate. The CC_{50} was calculated from the relation between surviving cells and drug concentration curve for each fraction using Sigmaplot software (Skehan, Storeng et al. 1990, Vajrabhaya and Korsuwannawong 2018).

UHPLC/Q-TOF-MS-MS analysis

The active fraction sample was analyzed at the Proteomics and Metabolomics lab, Cancer Children Hospital Egypt 57357, Cairo, Egypt and the sample was processed following Eissa et al procedure (Eissa, Hashim et al. 2020). Briefly, 50 mg of the sample was dissolved in 1 mL of deionized water, methanol, and acetonitrile mixture in a ratio of

50:25:25, respectively. The mixture was vortexed for 2 min followed by ultra-sonication for 10 min and centrifuged for 5 min at 10,000 rpm. 20 µl stock (50/ 1000 µl) was diluted with 1000 µl reconstitution solvent to obtain finally 1µg/µl injected concentration. 25 µl of prepared sample was injected on a positive mode. The phytoconstituents of the fraction were analyzed by using UHPLC/Q-TOF-MS MS in positive mode. 10 µL of the sample was injected and the used mobile phases were solvent A (5 mM ammonium format buffer pH 3 containing 1% methanol, and solvent B (100% acetonitrile). The gradient elution at a Flow rate of 0.3 mL/min was used as follow; isocratic 90% solvent A and 10% B (0–1 min), linear from 90% to 10% solvent A 90% , 10% B to 10% A and 90% B (1.1–20.9 min), isocratic 10% solvent A and 90% B (21–25 min), and finally, isocratic 90% solvent A and 10% B (25.1–28 min). The retention time and masses of the detected molecules were recorded by Peakview 2.2 software (SCIEX, USA) and MS-DIAL 3.70 software for data processing (Tsugawa, Cajka et al. 2015).

Many databases as Respect positive (2737 records), HDMB (Human Metabolome Database) and NIST (National Institute of Standards and Technology) libraries were used as references for the tentative identification of compounds. The identification was based on comparing their masses and fragmentation patterns with published compounds in literature.

Results and discussion

Assessment of cytotoxic activity of different extracts

Herein, in order to specify the most potent cytotoxic fraction of *B. edulis*, the total methanolic extract as well as different fractions were tested for its cytotoxic activity against MCF-7, HCT-116 and HepG2 cell lines using neutral red uptake assay. The 50% cytotoxic concentration (CC_{50}) determined from the dose-response curve are shown in table 1.

The results revealed that the total methanolic extract and different fractions of *B. edulis* exhibited cytotoxic activity against the tested cell lines. The CC50 values against the different cancer cell lines (MCF-7, HCT-116 and HepG2) were ranged between 4.19 ± 0.51 µg/mL and 25.24 ± 2.3 µg/mL which is acceptable level of plant extracts activity according to the National Cancer Institute (NCI) (Alonso-Castro, Villarreal et al. 2011).

Table 1: The CC_{50} values (µg/ml) of total methanolic extract and different fractions against the different cancer cell lines

No.	Tested sample	Tested cell lines (µg/mL)		
		MCF-7	HCT-116	HepG2
1	TM	25.24 ± 2.3	20.35 ± 2.1	18.27 ± 1.9
2	Hex	15.32 ± 1.57	12.22± 1.21	7.52 ± 0.82
3	DCM	10.78 ± 1.1	8.46 ± 0.86	6.25 ± 0.66
4	EtOAc	17.23 ± 1.68	19.12± 1.8	15.19 ± 1.52
5	But	9.12 ± 0.92	6.79 ± 0.65	4.19 ± 0.51
6	Dox	4.63 ± 0.30	7.25 ± 0.34	3.56 ± 0.61

TM, Total methanolic extract; Hex, *n*-Hexane fraction; DCM, Dichloromethane fraction, EtOAc, Ethyl acetate fraction; But, *n*-Butanol fraction; Dox, Doxorubicin.

Despite the high exhibited activity of hexane fraction against HepG2 (7.52 ± 0.82) and activity of dichloromethane fraction against HepG2 (6.25 ± 0.66) and HCT-116 (8.46 ± 0.86) while the *n*-butanol fraction showed the most potent cytotoxic activity against the three tested cancer cell line with CC_{50} values 9.12 ± 0.92, 6.79 ± 0.65 and 4.19 ± 0.51 against MCF-7, HCT-116 and HepG2 cell lines, respectively (Figure 1). further investigation using UPLC/Q-TOF-MS-MS technique was done to determine the constituents which may be responsible for the activity of *n*-butanol fraction.

Figure 1: The CC_{50} values of different fractions against the different cancer cell lines

Identification of active fraction phytoconstituents by UHPLC/Q-TOF-MS-MS

Recently, LC/Q-TOF-MS-MS technique represents an important fast reliable approach to identifying the constituents of plant extracts (Wolfender, Queiroz et al. 2006). The phytochemical constituents of *B. edulis n*-butanol as potent cytotoxic fraction were analyzed via UHPLC/Q-TOF-MS-MS in positive ionization modes. A total of 30 identified peaks belong to different classes including flavonoids (flavanones, flavonols, flavones, Isoflavonoids, chalcones and anthocyanins), nitrogenous compounds (alkaloids), and aromatic aldehydes and acids were tentatively identified, as shown in Table 2. The base peak chromatogram (BPC) of the analyzed sample is shown in Figure2.

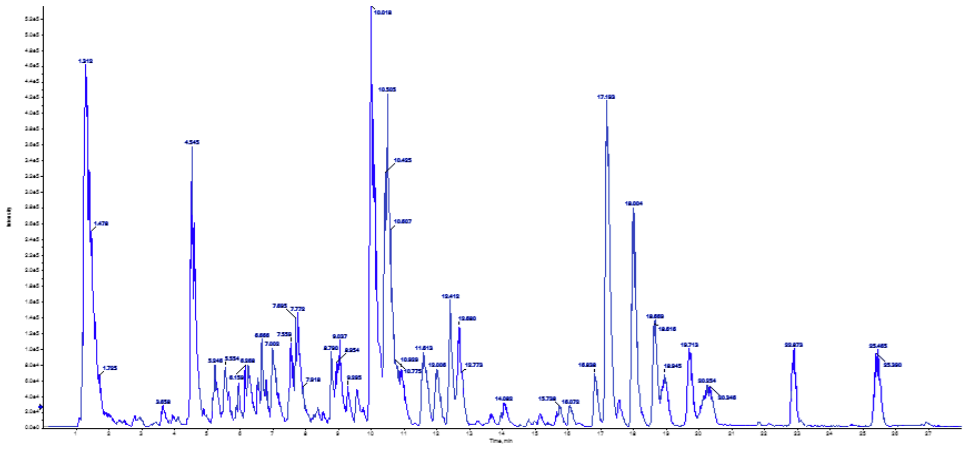

Figure 2: BPC of *n*-butanol fraction in positive ionization mode.

Identification of flavonoids

Most of identified compounds were flavonoids either *O*-glycosides (20 compounds) or aglycones (3 compounds) as protonated molecules $[M+H]^+$. The flavonoid glycosides characterized by elimination of the sugar residue, that is, 176 amu (hexuronic acid), 162 amu (hexose: glucose or galactose) and 146 amu (rhamnose) (Farag, El Fishawy et al. 2016). Fragmentation of flavonoid aglycones followed retro Diels-Alder (RDA) cleavage mechanism of C-ring also losing of 15, 18, 28 and 42 amu was commonly observed which representing (CH_3), (H_2O), (CO) and (CH_2CO) respectively (Tsimogiannis, Samiotaki et al. 2007) as shown in the table 2 and figure 3

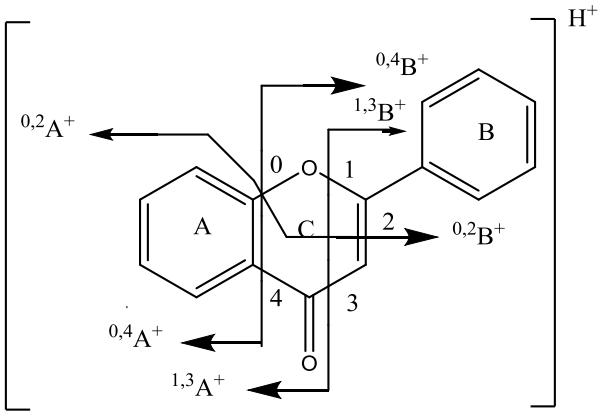

Figure 3: Schematic RDA diagram of ring C flavonoid, A and B represent the intact ring while the superscript on the left indicates the broken bonds of the protonated molecule.

The identified flavonoid constituents include flavones presented by compounds [apigenin-7-*O*-glucoside (**11**), diosmetin 7-*O*-rutinoside (**13**), baicalein-7-*O*-guluronide (**16**), diosmetin (**26**),and acacetin 7-*O*-neohespridoside (**27**)] and an isoflavone observed by compound biochanin A-7-*O*-glucoside (**22**) and flavanols identified compounds [isorhamnetin-3-*O*-rutinoside (**2**), Kaempferol-3- *O* -glucouronoid (**8**), eriodictyol-7-*O*-

glucoside (**9**), Kaempferol-3,7-*O*-bis-*α*-L-rahmnoside (**14**), and a Quercetin-4'- *O* -glucoside (**19**)] and flavanones presented by [eriodictyol-7-*O*-neohespridoside (**7**), and naringenin (**20**)] and anthocyanins identified by compounds [petunidin-3-*O*-ß -glucoside (**6**), pelargonidin-3,5- *O*- di-glucoside (**10**), cyanidine-3- *O* -glucoside (**12**), malvidin-3-*O*-glucoside (**18**), and cyanidine-3-*O*-rutinoside (**24**)] and chalcones detected by compounds [okanin-4'-*O*-glucoside (**15**), naringenin chalcone (**17**)] in addition to the aglycons which observed by compounds[luteolin (**21**), acacetin (**29**), apigenin (**25**)] (Figures 4)

Identification of aromatic aldehydes and acids

Investigation of the data revealed the presence of three aldehydes [cinnamaldehyde (**1**), syringaldehyde (**5**) and coniferaldehyde (**23**)] and two acids [caffeic acid (**28**) and *trans*-cinnamic acid (**30**)]. The neutral mass loss of hydroxyl, methyl, carboxylic, or aldehydic groups were helpful in identification as discussed in table 2 and figures 3&4.

Identification of nitrogenous compounds:

The analysis of a positive mode ESI results data revealed the presence of two alkaloids [Caffeine (**3**), and trigonelline (**4**)] were tentatively identified by comparison with previously published literature (Table 2, Figure 4). The analysis of the results showed that baicalein-7-*O*-glucuronide (**16**) was the major compounds with relative percent (28.12%) followed by Malvidin-3-*O*-glucoside (**18**), (23.84%). The previously published investigations of these compounds proved their anticancer activities against different of types cancer. baicalin (**16**) exhibited activity against prostate cancer cell lines (DU145) with IC_{50} 150 µM by (MTT) assay method (Wang, Wang et al. 2018), while, malvidin-3-*O*-glucoside (**18**), exerts their effect on breast cancer cell line (MCF-7) with IC_{50} 109.08 µM evaluated by SRB method (Oliveira, Wu et al. 2016). In addition, it was observed a promising activity against gastric cancer cell line (MKN-28) (Oliveira, Wu et al. 2016). This confirms that these compounds may be responsible for the cytotoxic activity of *n*-butanol fraction.

Table 2: List of identified compounds from *n*-Butanol fraction of *B. edulis*

No.	Proposed Compound	Rt (min)	Relative percentage	Precursor m/z (MS^1)	Adduct Ion	Chemical Formula	Error (ppm)	Characteristic Fragments (MS^2)	Ref
1	Cinnamaldehyde	3.42	0.0626	133.0626	$[M+H]^+$	$C_9H_9O^+$	10.9	105 $[M+H-CO]^+$	(Mohammed, Khan et al. 2021)
2	Isorhamnetin-3-*O*-rutinoside	3.78	0.0914	625.1853	$[M+H]^+$	$C_{28}H_{33}O_{16}^+$	-1	317 $[M+H\text{-rutinose}]^+$	(Zhou, Tang et al. 2014, Spínola, Pinto et al. 2015, Hefny Gad, Tuenter et al. 2018)
3	Caffeine	4.77	0.2540	195.0865	$[M+H]^+$	$C_8H_{11}N_4O_2^+$	-0.4	110 $[M+H\text{-methyl isocyanate-CO}]^+$, 138 $[M+H\text{-methyl isocyanate}]^+$	(Ismail, Manickam et al. 2000, Bianco, Abate et al. 2009) .
4	Trigonelline	5.75	0.6295	138.0552	$[M+H]^+$	$C_7H_8NO_2^+$	-1.8	79 $[M+H-CO_2-CH_3]^+$	(Lang, Yagar et al. 2013, da Rosa, Freitas-Silva et al. 2016)
5	Syringaldehyde	6.35	0.2074	183.0924	$[M+H]^+$	$C_9H_{11}O_4^+$	-0.1	95 $[M+H\text{-}C_3H_4O_3]^+$, 168$[M+H\text{-}CH_3]^+$	(Gioacchini, Roda et al. 1996, Flamini, Vedova et al. 2007, Thiyam, Claudia et al. 2009)
6	Petunidin-3-*O*-ß-glucopyranoside	6.51	2.1865	479.1165	$[M]^+$	$C_{22}H_{23}O_{12}^+$	1.8	302 $[M\text{-glucose-}CH_3]^+$, 317$[M\text{-glucose}]^+$	(Faria, Marques et al. 2011)
7	Eriodictyol-7-*O*-neohesperidoside (Neoeriocitrin)	6.54	0.7404	597.1531	$[M+H]^+$	$C_{27}H_{33}O_{15}^+$	6.4	288 $[aglycon]^+$, 435 $[M+H\text{-}C_6H_{10}O_5]^+$	(Mencherini, Campone et al. 2013, Spínola, Pinto et al. 2015)
8	Kaempferol-3-*O*-Glucuronide	6.75	14.2765	463.0888	$[M+H]^+$	$C_{21}H_{19}O_{12}^+$	-3	153 $^{1,3}A^+$, 241 $[M+H\text{- glucuronic acid -}H_2O\text{-CO}]^+$, 259 $[M+H\text{-glucuronic acid -CO}]^+$, 269 $[M+H\text{-glucuronic acid -}H_2O]^+$, 287 $[M+H\text{-glucuronic acid}]^+$	(Tsimogiannis, Samiotaki et al. 2007)
9	Eriodictyol-7-*O*-glucoside	6.82	0.3256	451.1225	$[M+H]^+$	$C_{21}H_{22}O_{11}^+$	0.1	153 $^{1,3}A^+$ 163 $^{0,4}B^+$, 179 $[M+H\text{-glucose -B-ring}]^+$, 289 $[M+H\text{-glucose}]^+$	(Tsimogiannis, Samiotaki et al. 2007, Peng, Lin et al. 2019)
10	Pelargonidin-3,5-*O*-di-glucoside	6.87	0.7378	595.1636	$[M]^+$	$C_{27}H_{31}O_{15}^+$	1.6	271 $[M\text{-2glucose}]^+$, 433 $[M\text{- glucose}]^+$	(Faria, Marques et al. 2011, Diretto, Jin et al. 2019) .
11	Apigenin-7-*O*-glucoside	6.88	0.3270	433.0893	$[M+H]^+$	$C_{21}H_{21}O_{10}^+$	0	271 $[M+H\text{-glucose}]^+$	(Petreska, Stefkov et al. 2011, Bergantin, Maietti et al. 2017)
12	Cyanidin-3-glucoside	6.91	1.2231	449.109	$[M]^+$	$C_{21}H_{21}O_{11}^+$	-1.7	287 $[M\text{-glucose}]^+$	(Lopes-da-Silva, de Pascual-Teresa et al.

No.	Proposed Compound	Rt (min)	Relative percentage	Precursor m/z (MS^1)	Adduct Ion	Chemical Formula	Error (ppm)	Characteristic Fragments (MS^2)	Ref
	(Kuromanin)								2002, Mena, Calani et al. 2012)
13	Diosmetin 7-*O*-rutinoside (Diosmin)	7.24	0.7930	609.1583	$[M+H]^+$	$C_{28}H_{33}O_{15}^+$	-0.9	463 [M+H-rhamnose]$^+$	(Gattuso, Barreca et al. 2007)
14	Kaempferol-3,7-*O*- α -alpha-L-rhamnoside	7.26	0.4519	579.1415	$[M+H]^+$	$C_{27}H_{31}O_{14}^+$	4.7	433 [M+H-rhamnose]$^+$	(Ghareeb, Saad et al. 2018, Santos, Fortunato et al. 2019)
15	Okanin-4'-*O*-glucoside (Marein)	7.29	0.3541	451.1671	$[M+H]^+$	$C_{21}H_{23}O_{11}^+$	11.9	289 [M+H-glucose]$^+$	(Yang, Sun et al. 2016, Peng, Lin et al. 2019)
16	Baicalein-7-*O*-glucuronide (Baicalin)	7.55	28.1208	447.0919	$[M+H]^+$	$C_{21}H_{19}O_{11}^+$	0.3	271 [M+H-glucuronic acid]$^+$	(Chung, Lim et al. 2012)
17	Naringenin chalcone	7.73	4.6058	273.0728	$[M+H]^+$	$C_{15}H_{13}O_5^+$	8.1	153 [M + H-C_8H_8O]$^+$	(Wani, Pandith et al. 2017)
18	Malvidin-3-*O*-glucoside	7.75	23.8477	493.1318	$[M]^+$	$C_{23}H_{25}O_{12}^+$	4	331 [M-glucose]$^+$	(Ştefănuţ, Căta et al. 2011)
19	Quercetin-4'-glucoside (Spiraeoside)	7.96	1.5639	465.1359	$[M+H]^+$	$C_{21}H_{21}O_{12}^+$	0.8	303 [M+H-glucose]$^+$	(Bonaccorsi, Caristi et al. 2008)
20	Naringenin	8.99	0.2905	273.0749	$[M+H]^+$	$C_{15}H_{13}O_5^+$	2.5	153 [M + H-C_8H_8O]$^+$	(Wani, Pandith et al. 2017, Peng, Lin et al. 2019)
21	Luteolin	9.66	2.0681	287.0537	$[M+H]^+$	$C_{15}H_{11}O_6^+$	0.1	135 $^{1,3}B^+$, 153[M + H-$C_8H_6O_2$]$^+$, 269[M+H-H_2O]$^+$	(Tsimogiannis, Samiotaki et al. 2007, Yang, Sun et al. 2016, Peng, Lin et al. 2019)
22	biochanin A 7-*O*-glucoside (Sissotrin)	9.80	0.4987	447.1241	$[M+H]^+$	$C_{22}H_{23}O_{10}^+$	-2	270 [M+H-glucose-CH_3]$^+$ 285 [M+H-glucose]$^+$	(Stobiecki, Staszków et al. 2010, Roriz, Barros et al. 2014)
23	4-Hydroxy-3-methoxycinnamaldehyde (Coniferaldehyde)	10.35	1.5369	179.0699	$[M+H]^+$	$C_{10}H_{11}O_3^+$	-0.3	119 [M+H-CH_3OH-CO]$^+$, 147 [M+H-CH_3OH]$^+$	(Flamini, Vedova et al. 2007)
24	Cyanidin-3-*O*-rutinoside	10.38	4.1634	595.1433	$[M]^+$	$C_{27}H_{31}O_{15}^+$	0.8	287 [M-rutinose]$^+$	(Kallam, Appelhagen et al. 2017)
25	Apigenin	10.84	3.6397	271.0585	$[M+H]^+$	$C_{15}H_{11}O_5^+$	2.6	119 $^{1,3}B^+$, 121 $^{0,2}B^+$, 153 $^{1,3}A^+$, 225 [M+H-H_2O-CO]$^+$	(Tsimogiannis, Samiotaki et al. 2007)
26	(3, 5 ,7-trihydroxy-4'-methoxyflavone) (Diosmetin)	11.14	2.8812	301.0702	$[M+H]^+$	$C_{16}H_{13}O_6^+$	1	153 $^{1,3}A^+$, 229 [M+H-CH_3-COH-CO]$^+$, 257 [M+H-CH_3-COH]$^+$	(Fridén and Sjöberg 2014, Peng, Lin et al. 2019)

No.	Proposed Compound	Rt (min)	Relative percentage	Precursor m/z (MS^1)	Adduct Ion	Chemical Formula	Error (ppm)	Characteristic Fragments (MS^2)	Ref
27	Acacetin-7-*O*-neohesperidoside (Fortunellin)	11.78	0.3369	593.1643	$[M+H]^+$	$C_{28}H_{33}O_{14}^+$	-0.8	285 [M+H-neohesperidose]$^+$ 431[M+H-$C_6H_{10}O_5$]$^+$	(Feng, Liu et al. 2014)
28	Caffeic acid	12.69	1.3625	181.1200	$[M+H]^+$	$C_9H_9O_4^+$	2.5	145 [M+H-$2H_2O$]$^+$, 153 [M+H-CO]$^+$, 163 [M+H-H_2O]$^+$	(Wu, Ma et al. 2009, Bergantin, Maietti et al. 2017)
29	Acacetin	13.83	0.1245	285.0741	$[M+H]^+$	$C_{16}H_{13}O_5^+$	4.5	153 [M+H-C_9H_8O]$^+$, 242 [M+H-CH_3-CO]$^+$	(Peng, Lin et al. 2019)
30	trans-Cinnamic acid	14.26	2.2975	149.0221	$[M+H]^+$	$C_9H_9O_2^+$	4.3	121 [M+H-CO]$^+$	(Sun, Liang et al. 2007, Baeza, Sarriá et al. 2016)

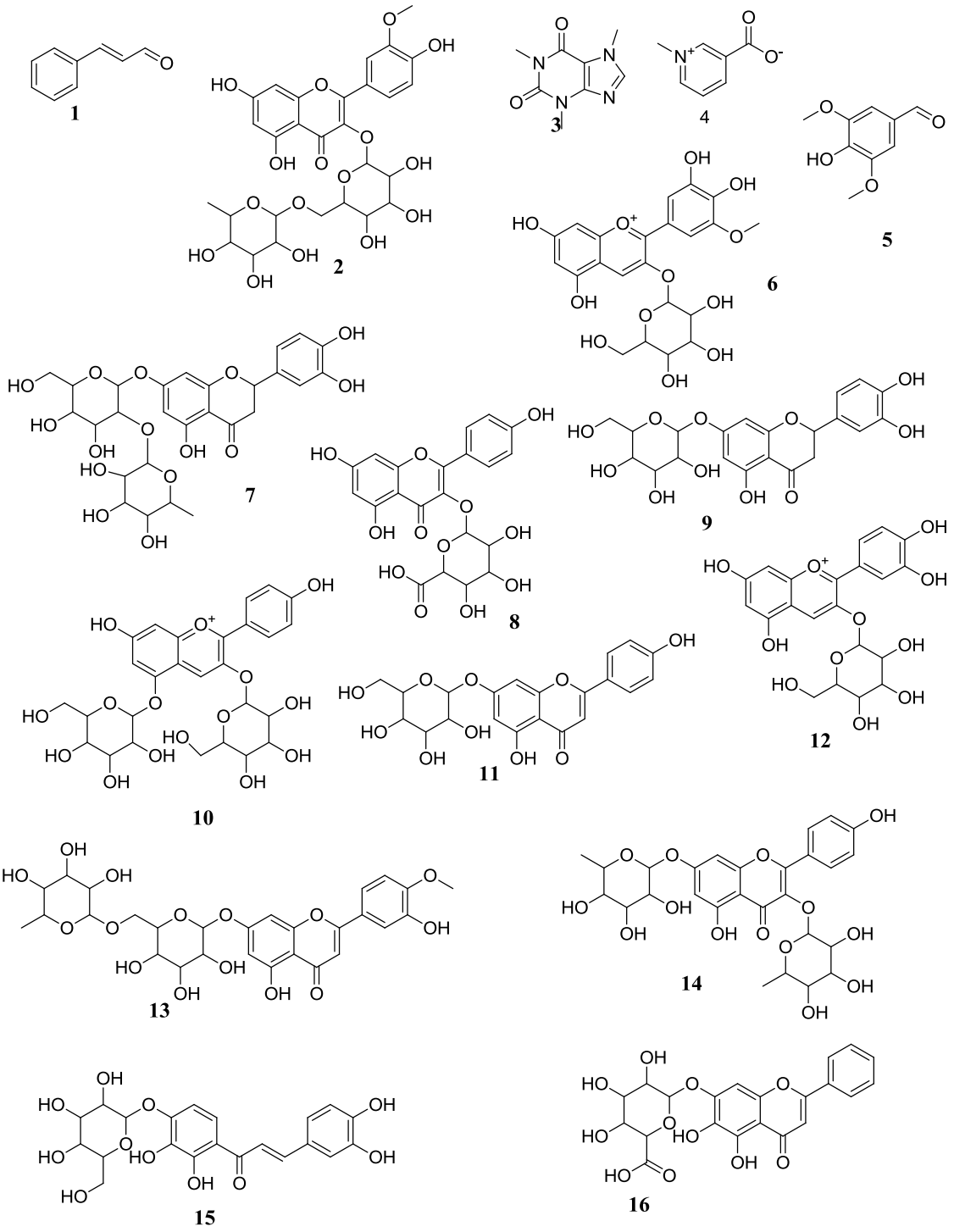

Figure 3: Structures of identified compounds (**1-30**)

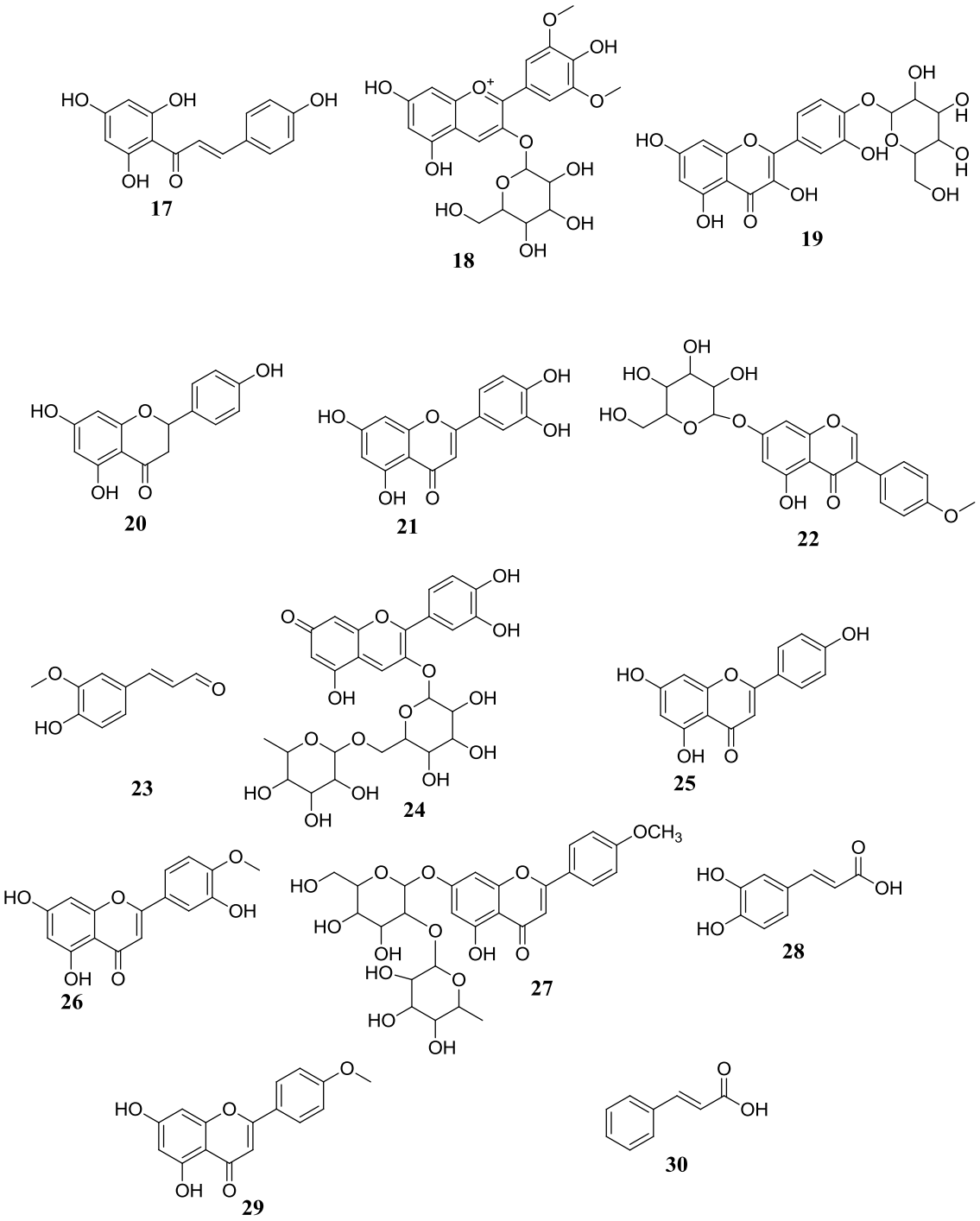

Figure 3: Structures of identified compounds (**1-30**)

Conclusion

This study shed light on cytotoxic activities of different fractions of *B. edulis* methanolic extract against Human colon carcinoma (HCT-116), Human colon carcinoma (MCF-7) and Hepatocellular carcinoma ($HepG_2$) cell lines. Despite the different fractions showed promising activity, the n-butanol fraction was the most potent one. Metabolites profiling of the potent *n*-butanol fraction using UHPLC/Q-TOF-MS-MS analysis was conducted. The results analysis showed that baicalein-7-*O*-glucuronide and malvidin-3-*O*-glucoside were the major identified compounds. The promising results in the current study suggests further investigations for *B. edulis* phytochemicals and biological activities.

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التعرف على المركبات الفيتوكيميائية لنبات بليفارس ايديوليس فروسك بيرز باستخدام جهاز الفصل الكروماتوجرافى فائق الكفاءة المقترن بمطياف الكتلة وفاعليته ضد الخلايا السرطانية

طه عبد المعبود[١]* ، مصطفى حجازى[١] ، عبد السلام ابراهيم[١] ، لطفى دياب[١]

[١]قسم العقاقير ،كلية الصيدلة ،جامعة الازهر ،القاهرة ،مصر

***البريد الالكتروني للباحث الرئيسي :** TahaKedra.2@azhar.edu.eg

الملخص :

في الآونة الأخيرة ازداد الطلب على نطاق واسع لاكتشاف عقاقير امنة مضادة للسرطان من الطبيعة تهدف الى تجنب الاثار الجانبية للعلاج الكيميائى. تعتبر عشبة البليفاريس ايديوليس فروسك. بير.عشب دائم ينمو فى مصر يتبع عائلة الاكانسيسيا وله العديد من الانشطة البيولوجية الشعبية والدوائية. وتم فى هذه الدراسة اختبار سمية الخلاصات المختلفة ضد ثلاثة خلايا سرطانية باستخدام صبغة سلفورودامين ؛ خلايا سرطان الخلايا الكبدية، وخلايا سرطان القولون البشري، وخلايا سرطان الثدى البشري وكشفت النتائج أن مستخلص البوتانول أظهر أقوى نشاط ضد الخلايا السرطانية الثلاث المختبرة بقيم ٩.١٢ ± ٠.٩٢ و٦.٧٩ ± ٠.٦٥ و٤.١٩ ± ٠.٥١ على وخلايا سرطان الثدى البشري ، وخلايا سرطان القولون البشري، وسرطان الخلايا الكبدية الخلايا على التوالي. وعلاوة على ذلك، فإنه قد تم عمل التحليل الأيضي للمركبات الفيتوكيميائية بواسطة جهاز الفصل الكروماتوجرافى فائق الكفاءة المقترن بمطياف الكتلة لهذه الخلاصة الفعالة لتحديد المركبات النشطة والطبيعية والمسئولة عن فاعلية خلاصة البيوتانول كأكثر العينات نشاطا لأول مرة. هذا وقد تم التعرف على ثلاثون مركبا من مختلف الفئات الفيتوكيميائية بما في ذلك الفلافونويد والألديهيدات والأحماض الفينولية والقلويدات من نبات البليفارس ايديوليس

الكلمات المفتاحية : بليفارس ايديوليس ، اكانسيسيا ، النشاط السام للخلايا ، جهاز الفصل الكروماتوجرافى فائق الكفاءة المقترن بمطياف الكتلة